Delivery service

Liposomes are useful vehicles for transporting active ingredients into the skin. Gabriele Blume of Rovi presents some new studies on their penetration capability

Courtesy: Dead Sea Laboratories

Rovisomes are lipidic vesicles (liposomes) with the ability to penetrate into the skin and to act as a carrier system for different active ingredients. Various studies were carried out to prove these claims. With the help of Professor A Fahr of the University of Marburg, *in vivo* studies with carboxyfluorescein were undertaken.

Carboxyfluorescein (CF), a fluorescence dye with a molecular weight of 376Dt, was dissolved in water and encapsulated in the aqueous compartment of Rovi liposomes. These Rovisomes CF were applied non-occlusively onto the skin (10µL/cm^2), along with two control preparations of CF solution and CF solution with an emulsifier (2.5% Lanette E). After one hour the penetration profiles of CF were determined by tape stripping the skin and the amount of CF in each strip was measured by fluorescence spectroscopy.[1]

Figure 1 shows that, compared with the non-encapsulated carboxyfluorescein, penetration into the deeper skin layers was significantly enhanced when CF was entrapped in liposomes (pink). Water soluble CF was only found in the first five

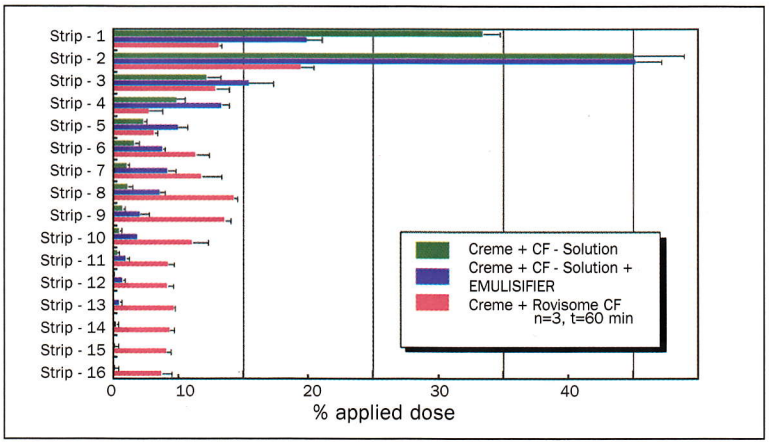


Figure 1 - Penetration profile of CF in its free form or encapsulated in liposomes

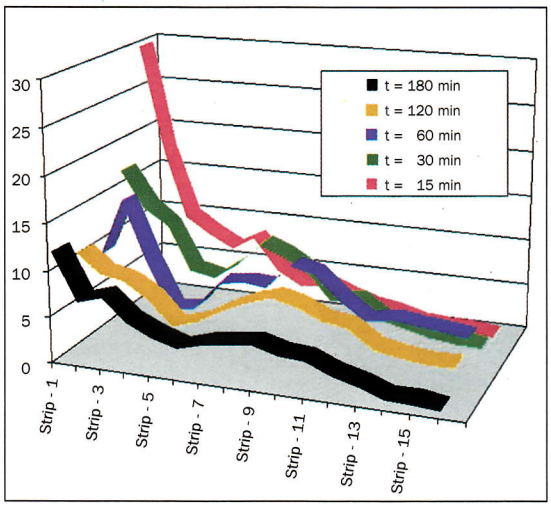


Figure 2 - Kinetic properties of Rovisomes containing CF

layers (green). The addition of an emulsifier to the CF-solution also led to a slight improvement in penetration, with CF being recovered up to layer 8 (purple). In contrast, the distribution profile of liposomal CF showed two peaks. The first peak spans layers 1-5 with 30% of the applied dose, correlating with the amount of dye not encapsulated into the liposomes. The second peak covers layers 7-13 with 70% of the applied dose transported into deeper skin layers by means of the carrier system.

In a second study the kinetic properties of Rovisomes with carboxyfluorescein was determined (figure 2). Just 15 minutes after application, it was possible to detect an accumulation of CF between strips 6-10, indicating the presence of liposomally encapsulated dye. In time, the carboxyfluorescein content transported with the liposomes moved in a wave-like pattern to the deeper skin layers. After three hours, only 70% of the dose originally applied was still to be found in the 16 strips. In contrast, free CF applied to the skin in a solution with an emulsifier was detected in only the first ten strips after a period of three hours (data not shown).

The aim of the next study (Figure 3) was to test the penetration behaviour of a day cream (o/w cream) mixed with a CF solution, unfilled liposomes and CF solution or Rovisome CF. Before application, the preparations were stored for one day. In the final analysis, all the preparations contained the same concentration of fluorescence dye. Application points were marked (1cm²) and 20µL doses of the individual preparations were carefully applied to these areas using a pipette. The quantity applied was doubled as the cream has a quenching effect on fluorescence.

The test shows that, in a cream, 70% of a water soluble ingredient stays in the top three strips (green). As with the previous variant, even in the presence of liposomes, approximately 75% of the free water soluble substances remained in these top three strips (purple). This reconfirms the fact that liposomes do not work as penetration boosters. In fact, the penetration level of CF nestling amidst the cream is actually lower than that of the free water soluble substance. One hour after application, approximately 50% was still to be found in the top three skin layers. The remaining 50% has characteristically penetrated through to strip 15. Some 25% of the water soluble substance can already be found in strips 9-15, whereas the multicomponent nature of the cream slows down penetration. This is due to the hydrophilic part of the cream preventing evaporation, which is actually the main cause of the liposome entering the skin.

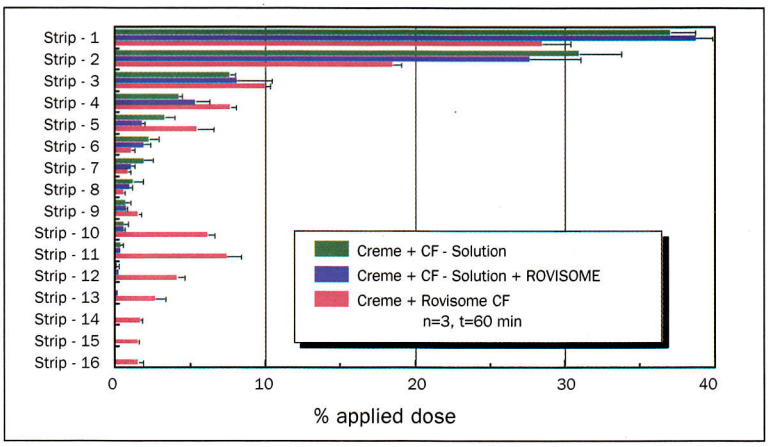


Figure 3 - Penetration profile of CF from a final formulation

Lipid composition

New *ex vivo* experiments with flurorescence dyes conducted by Dr U Schüfer at the University of Saarbrücken demonstrate the penetration profile of liposomal CF according to the lipid composition of the vesicles. In this study CF was non-occlusively applied onto human skin from surgery both in an ethanolic solution and encapsulated in three types of liposomes. The lecithins used for these liposomes differ in their phospholipid composition. Rovisomes are made of lecithin containing >75% of unsaturated phosphatidylcholine (PC), liposomes PL 25 are produced from phospholipids with a content of only 25% PC while liposomes PL 90H are made of lecithin containing >85% of saturated PC. There were no differences in the size of these vesicle preparations (120-160nm). The skin was placed in Franz-type diffusion cells and the acceptor compartment was filled with buffer below the skin to avoid over-hydration. Three hours after application the skin surface was cleaned and skin cylinders were punched. After cryofixation, pieces with a thickness of 10µm were cut and examined by confocal laser scanning miscroscopy (Bio Rad MRC 1024).

CF-solution applied onto the intact skin surface shows only a very limited skin penetration

capability due to the high amount of ethanol. The results of water soluble materials are normally even worse. In contrast, Rovisomes with their flexible membrane penetrate the horny layer and transport the water soluble CF into the epidermis. Liposomes in a gel state (PL 90H) penetrate the skin poorly while liposomes made of PL 25 are fixed on the skin surface (Figure 4).

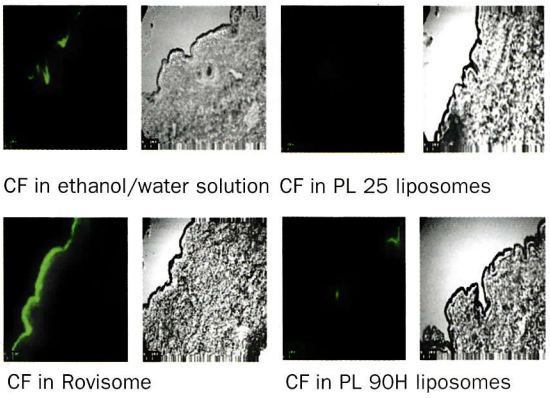
Rovisome E-acetate

In collaboration with Dr A Vierheilig of Nimbus and Henkel's Dr W Pitterman, studies were undertaken to examine the penetration behaviour of the lipophilic vitamin E (E-acetate) in a solution with an emulsifier or encapsulated in Rovisomes (Figure 5).The preparations were applied to the forearms of four volunteers. After one hour, the penetration profiles were examined by tape stripping the skin and measuring the vitamin content of each strip by ATR-FTIR (attenuated time resolved infrared spectroscopy).[2]

The skin builds up a reservoir of the applied pure vitamin and keeps the active ingredient in the first 4-5 layers of the stratum corneum. With the Rovisome carrier system, the vitamin is also transported in a significantly higher concentration to the deeper skin layers. These results correspond well with the penetration behaviour of water soluble magnesium-ascorbylphosphate.[3]

In collaboration with Henkel, formulations with vitamin E-acetate and Rovisome E-acetate were tested for their ability to penetrate the skin of isolated, perfused bovine udder.[4] In three independent studies the vitamin was applied either in a lamellar cream formulation or in a liposomal formulation (3-4g/100cm2) onto the bovine udder. After 30 minutes the skin areas were cleaned and for the detection of the substance adhesive tape stripping (15 strips) were routinely used. The amount of vitamin in the strips was measured by HPLC.

The naturally occurring concentration of vitamin E in the skin of non-treated udder amounts to 0.056µg vitamin E per cm^2



(15 strips). Only in the first strip was 50% of applied dose detected. The proportion of the vitamin content in strip 1 and the other 14 strips was found to be almost the same for the applications of the lamellar cream (42%/58%) as for the Rovisome E-acetate formulation (44%/56%). This means that there is the same distribution pattern of vitamin E in the skin layers regardless of the treatment.

However, there are significant differences in the amount of vitamin E-acetate found in each strip

Figure 4 - Penetration profile of CF encapsulated in different liposomes

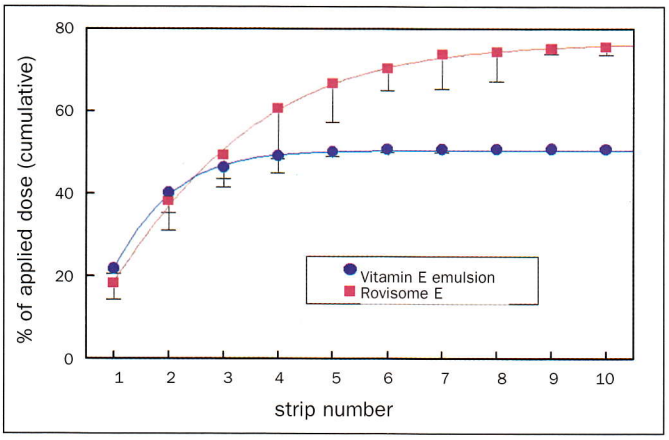


Figure 5 - Penetration profile of vitamin E in its free form or encapsulated in liposomes

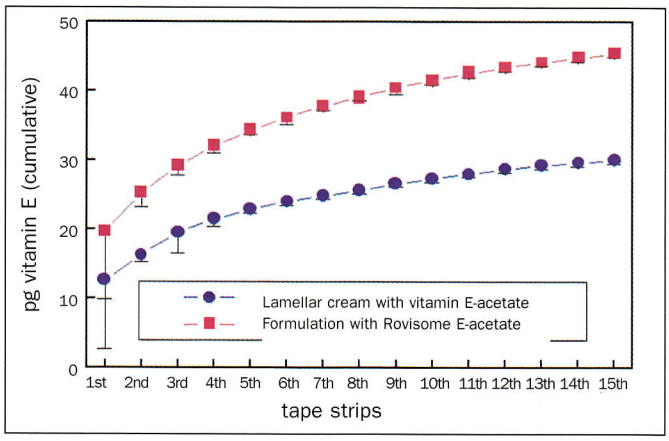


Figure 6 - Penetration profile of vitamin E from a final formulation

according to the applied formulation. Vitamin E encapsulated in Rovisomes was detected to have a total amount of 45.35µg of the vitamin in the 15 strips compared with 30.04µg for the lamellar cream.

Rovisomes are thus an ideal, stable system for transporting both lipophilic and water soluble substances into the skin and for making these active ingredients bio-available to the target sites. Furthermore, they are ideal for incorporation into a multitude of formulations, as well as being characterised by their own positive effects on the skin.

References
1. Cordech L, et al, Int J Pharm, 139, 197-203 (1996)
2. Vierheilig A & Braunschweig T, Parfümerie und Kosmetik Nr; 1-2, 20-21 (1998)
3. Blume G et al, SÖFW, 2, 298-301 (1997)
4. Pittermann W, Parfümerie und Kosmetik, 3, 38-41 (1999)

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